

Preadmission Screening of Adults Highlights Previously Unrecognized Carriage of Panton-Valentine Leukocidin-Positive Methicillin-Resistant *Staphylococcus aureus* in London: a Cause for Concern?

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In the first study of its kind in the United Kingdom, we describe the colonization rate of ciprofloxacin-sensitive Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* (PVL-MRSA) in adult patients who were screened systematically at the time of hospital admission. We also describe the molecular characteristics of PVL-MRSA and antibiotic resistance phenotypes. A total of 55,760 specimens were screened for MRSA between April 2008 and December 2010. MRSA was identified in 1,998 specimens, and ciprofloxacin-susceptible (CSMRSA) isolates (385/1,998, or 19.3%) were subjected to PVL testing. Of these, 70 (18.1%) were identified as PVL-CSMRSA. During the study period, the MRSA colonization rate decreased from 4.6% to 2.8%. In contrast, the colonization rate of PVL-CSMRSA increased over time, rising from 0.075% in 2008 and 0.07% in 2009 to 0.22% in 2010. The mean patient age was 52 years (range, 18 to 90 years); over two-thirds were male. Seven different lineages of PVL-CSMRSA were identified. Over the 3 years, the Southwest Pacific clone (CC30) was dominant in our population. The CC5 clone was detected once in 2008 and not at all in 2009 but accounted for a third of all PVL-CSMRSA strains in 2010. This lineage was commonly associated with clindamycin resistance and, less frequently, tetracycline resistance. We conclude that there is hitherto unrecognized low-level carriage of PVL-CSMRSA among patients being admitted to hospitals in northwest London. We observed the emergence of the CC5 clone in 2010 with associated clindamycin and tetracycline resistance.

Panton-Valentine leukocidin (PVL) is a cytotoxin that causes leukocyte destruction and necrosis of skin and mucosa. The PVL-encoding bacteriophages have been identified in multiple lineages of *Staphylococcus aureus* (PVL-SA), enhancing their virulence and the ability to cause both community- and hospital-acquired infections (12, 18, 25).

Typically, PVL-SA infections manifest as pyogenic skin and soft tissue infections, requiring antibiotic treatment and/or incision and drainage (15). In a minority of cases, they cause more invasive disease, the most serious of which is necrotizing hemorrhagic pneumonia, which has a high mortality rate (8).

In March 2005, the Health Protection Agency (HPA) reported that PVL methicillin-resistant *S. aureus* (PVL-MRSA) was an emerging issue in England (10). While more recent data point to the recognition of multiresistant PVL-MRSA (6), early strains were believed to be nonmultiresistant, and susceptibility to ciprofloxacin was used as a putative marker of PVL-MRSA. Accordingly, diagnostic laboratories were alerted to consider PVL testing of nonmultiresistant MRSA (in particular, ciprofloxacin-susceptible strains). Data from the HPA Staphylococcus Reference Unit (SRU) from 2005 to 2010 showed a 2-fold increase in the number of PVL-SA cases identified annually in England (9). The majority were with methicillin-sensitive *S. aureus* (PVL-MSSA), although methicillin-resistant strains (PVL-MRSA) also appear to be increasing in prevalence and account for an increasing proportion of the total PVL-SA in the United Kingdom (9). The data reported by the HPA were from isolates derived from clinical specimens referred from diagnostic microbiology laboratories in England on a voluntary basis; there is no mandate for isolates to be submitted for testing. It is therefore unclear whether this increase is genuine or due to increased referral to the reference laboratory. In a recent

study, Ellington et al. recommended planned systematic studies to address this question (6).

It is recognized that colonization often precedes infection and that increased prevalence of colonization is associated with a greater number of infections (16, 22). Thus, it may be argued that prevalence of colonization provides a better measure of the distribution and burden of PVL-MRSA in the population. Little is known about the carrier state of PVL-MRSA in emergency admissions, which may represent a hidden community reservoir and potential for introduction into health care settings.

In this paper, we describe the colonization rate of ciprofloxacin-susceptible PVL-MRSA colonization in adult patients (>17 years) who were systematically and nonselectively screened at the time of hospital admission. We also describe the molecular characteristics of PVL-CSMRSA and antibiotic resistance phenotypes to provide insights into their clonal diversity and associated antibiotic resistance.

MATERIALS AND METHODS

We conducted a prospective observational study at the North West London Hospitals (NWLH) NHS Trust between April 2008 and December 2010. NWLH consists of three hospitals: Northwick Park, Central Middle-

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TABLE 1 Trends in detection of PVL-MRSA in the period 2008–2010

Parameter ^a	Result			Chi-squared <i>P</i> values		
	2008	2009	2010	Overall	Linear trend	Nonlinear trend
No. of MRSA screens performed	14,571	21,354	19,835			
No. of MRSA strains isolated (%; 95% CI)	675 (4.6, 4.3–5.0)	763 (3.6, 3.3–3.8)	560 (2.8, 2.6–3.1)	<0.0001	<0.0001	0.3
No. of ciprofloxacin-susceptible MRSA strains (%; 95% CI)	70 (10.4, 8.1–12.7)	121 (15.9, 13.3–18.5)	194 (34.6, 30.7–38.6)	<0.0001	<0.0001	0.0003
No. of PVL-MRSA strains identified (%; 95% CI)	11 (15.7, 7.2–24.2)	15 (12.4, 6.5–18.3)	44 (22.7, 16.8–28.6)	0.06	0.07	0.12
Overall extrapolated prevalence of PVL CSMRSA [% (95% CI)]	0.075 (0–0.1)	0.070 (0–0.1)	0.220 (0.2–0.3)	<0.0001	0.0001	0.01
Overall percentage of MRSA strains that were PVL ⁺ (95% CI)	1.63 (0.7–2.6)	1.97 (1–3)	7.86 (5.6–10.1)	<0.0001	<0.0001	0.001

^a 95% CI, 95% confidence interval.

sex, and St. Mark's; the first two are busy district general hospitals, and the third is a tertiary referral center for colorectal disease.

Routine MRSA screening was undertaken for all emergency and elective adult admissions to the hospitals. Nose and groin swabs were taken to screen for MRSA either in the emergency department, at the time of admission to the ward, or at the preoperative assessment clinics. Swabs were pooled, inoculated onto a selective chromogenic medium for MRSA (Brilliance agar; Oxoid, United Kingdom), and incubated in air at 37°C for 18 to 24 h. All presumptive MRSA colonies were confirmed using a staphylococcal latex agglutination test (Staphylect Plus; Oxoid, United Kingdom). Susceptibility to a range of antibiotics was determined using the method recommended by the British Society of Antimicrobial Chemotherapy (BSAC method) (2). Inducible resistance to clindamycin was determined using the double disk diffusion D test. Ciprofloxacin-susceptible MRSA strains were referred to the SRU for detection of PVL-encoding genes. PVL-CSMRSA strains were further characterized by testing for *mecA*, toxin gene profiling (for 14 toxin genes), *spa* typing, and/or pulsed-field gel electrophoresis of total DNA restricted with *Sma*I as described previously (7).

Only one isolate per patient was included in the PVL-CSMRSA aspect of this study. Demographic data of the patients were obtained from laboratory information systems (LIS).

Differences in the proportions of MRSA and PVL-CSMRSA between the three study years were assessed by partitioning the chi-square test of association into tests for linear and nonlinear trends. Logistic regression was performed to provide direct comparisons in proportions between

years. Temporal trends in the multilocus sequence typing clonal complex (MLST-CC) lineages were assessed using Poisson regression. *P* values of 0.05 or lower were considered as indicating statistical significance.

RESULTS

A total of 55,760 specimens were screened for MRSA between April 2008 and December 2010. MRSA was identified in 1,998 specimens (3.6%); of these, 385 (0.7%) were CSMRSA. The latter were subjected to PVL testing, and 70 (extrapolated prevalence of 0.12%) were identified as PVL-CSMRSA.

During the study period, the MRSA carriage colonization rate significantly decreased by around 25% each year ($P < 0.0001$), from 4.6% in 2008 to 2.8% in 2010. The colonization rates of PVL-CSMRSA were similar in 2008 (0.075%) and 2009 (0.070%) ($P = 0.9$, logistic regression), but the rate increased significantly in 2010 (0.22%; $P < 0.05$) ($P = 0.001$, logistic regression comparing 2010 to 2008) (Table 1).

The genotypic characteristics of the PVL-CSMRSA and their associated resistance phenotypes are summarized in Table 2. There was a significant temporal trend over the 3 years in the number of CC5 isolates ($P = 0.002$, Poisson regression); the other two main genotypes had only weak evidence of increasing numbers ($P = 0.1$ and $P = 0.13$ for CC22 and CC30, respectively). The other genotypes were too small to assess genotype-specific trends,

TABLE 2 Phenotypic and genotypic characteristics of PVL-CSMRSA (2008–2010)

MLST-CC lineage ^a	<i>spa</i> type(s)	No. identified by year			Common name	No. of isolates resistant ^b					
		2008	2009	2010		GEN	TET	FA	RIF	CLIN	CLR
CC30	t019, t122	7	11	14	Southwest Pacific clone	1				1	2
CC5	t002, t088	1		14			2			11	11
CC22	t005, t891	2	4	7		2				3	3
CC80	t044	1	1	6	European clone			7		1	1
CC8	t008		1	1	USA300 clone		2				2
CC59	t441			1	Southeast Asia clone					1	1
CC152	t355			1		1					

^a Inferred from *spa* repeat succession data.

^b Some isolates exhibited resistance to more than one antibiotic. Isolates sensitive to gentamicin (GEN), tetracycline (TET), fusidic acid (FA), rifampin (RIF), clindamycin (CLIN), and clarithromycin (CLR) are not included in the section of resistant isolates. Thus, the total number of isolates does not correspond to the total number of CSMRSA isolates identified in the study period.

but when they were combined, there was a significant increase in numbers over the 3 years ($P = 0.01$).

The mean age of patients colonized with PVL-CSMRSA was 52 years (range, 18 to 90 years); over two-thirds were male ($P = 0.002$, Poisson regression).

DISCUSSION

The most notable PVL-MRSA lineage is the USA300 clone (ST8-IVa), which first emerged as a public health concern among college football players in the United States in 2000 (26). Over the subsequent decade it has reached epidemic proportions: it is now the predominant community-associated MRSA strain (CA-MRSA) in North America and is endemic within many U.S. hospitals (14). There is a clear need for public health professionals to remain alert to prevent this trend being mirrored on other continents.

In England, PVL-MRSA has not followed the same epidemic curve as that observed in the United States; rather than a single PVL-MRSA clone predominating, multiple lineages have been identified and are generally considered to be relatively rare, although the true prevalence remains largely unknown (7). The majority of PVL-MRSA cases are community associated. In 2007, a study undertaken in a North London hospital showed that only 0.8% of 394 consecutive MRSA isolates from clinical specimens were PVL positive (24).

We have previously reported a PVL-MRSA carriage rate of 0.06% among adult emergency admissions, none of whom had clinical infections with PVL-MRSA (13).

To the best of our knowledge, this is the first study in the United Kingdom to report the colonization rate of PVL-CSMRSA in adults through systematic screening of all adult hospital admissions over a 3-year period (2008 to 2010) including over 55,000 patients. Fewer patients were screened in 2008 than in 2009 and 2010 because the policy for screening all adult hospital admissions was introduced in April 2008. As the policy required unselective screening of all patients, we are confident that there was no systematic bias. We have demonstrated that, among ciprofloxacin-susceptible MRSA, PVL-CSMRSA has increased in prevalence in the local community over this time, most notably in 2010. Given that the patients were screened at the time of hospital admission, this may reflect a changing prevalence in the community. Currently, hospital-acquired PVL-MRSA is rare in England (1, 18, 25). However, the rising colonization rate in patients admitted to our hospital demonstrates a potential for nosocomial transmission, as has been observed with the USA300 clone in North America, where it is now recognized as a common cause of hospital-acquired infection (14, 19–21).

In this study, PVL-CSMRSA colonization was more commonly observed in males. This is consistent with previous reports that male sex is a risk factor for carriage of *S. aureus* and clinical infections with PVL-MRSA (23, 24).

As patients under the age of 18 years were not screened, we are unable to comment on the colonization rate of PVL-CSMRSA in this age group. However, we did observe colonization in a wide range of ages, including some elderly patients, confirming that PVL-CSMRSA is not restricted to children and young adults.

Molecular epidemiological data show that at least 7 different lineages of PVL-CSMRSA were identified in this study. This clonal diversity echoes previous reports of PVL-MRSA identified in England and strongly suggests a community origin (7).

However, the clonal diversity of the PVL-CSMRSA detected in this study needs to be confirmed by broader community-based prevalence studies.

Over the 3 years, the Southwest Pacific clone (CC30-IVc) was the dominant lineage seen in our population. The CC5 lineage was detected only once in 2008 and not at all in 2009, but it accounted for a third of all PVL-CSMRSA cases in 2010. There was no evidence of clonal expansion within this lineage: based on phenotypic and genotypic analyses (*spa*, pulsotype, and toxin gene profiling [data not shown]), multiple strains were identified. Of note, the majority of CC5 strains were resistant to clindamycin, and two were also resistant to tetracycline. The emergence of clindamycin resistance and its association with clonal shift have been described previously (3–5). Clindamycin and tetracycline are currently recommended in the HPA's guidance for the management of PVL-associated *Staphylococcus aureus* infections (PVL-SA) in England (11).

Thus, the emergence of resistant lineages such as CC5 restricts further the options available for treatment. As described previously, fusidic acid resistance was almost universally present in CC80 isolates (European clone) (17). We did not observe resistance to rifampin, suggesting that this agent is likely to be useful in the empirical treatment of suspected PVL-MRSA infections. However, it is well recognized that the use of rifampin as a sole agent for treatment leads to rapid emergence of resistance and, therefore, it should be used in combination with another antibiotic(s).

It is important to acknowledge that this study has some limitations. We have determined the colonization rate of only ciprofloxacin-sensitive PVL-MRSA. We would have excluded/underestimated ciprofloxacin-resistant lineages which are known to occur in England (e.g., Bengal Bay [ST772], USA300, CC22, and South-east Asia [CC59] clones) (7). As no routine MRSA screening is undertaken with children in our hospitals, we could not estimate the colonization rate of PVL-CSMRSA colonization in this group. Finally, as the demographic data are derived from the LIS, we were limited by the ability of the system to deduplicate the records of all the patients screened for MRSA although we were able to do so for patients with PVL-CSMRSA.

Conclusion. Through routine preadmission screening of adults over a period of 3 years, we have shown that PVL-CSMRSA colonization is increasing in prevalence in our local population in all ages, especially in males. Furthermore, although the current prevalence is relatively low, there is a potential for PVL-MRSA to infiltrate health care facilities and result in nosocomial transmission/health care-associated infections. We are currently investigating the occurrence and clonal diversity of hospital-acquired PVL-MRSA infections; the results of this study will be published separately.

We have confirmed that diverse clones of PVL-CSMRSA are circulating in the community. The emergence of CC5 in 2010 and its association with clindamycin and tetracycline resistance are likely to have important implications for empirical treatment of suspected PVL-CSMRSA infections.

Our findings emphasize the need to be vigilant and establish more systematic surveillance for PVL-MRSA to monitor their prevalence, evolution, and pathogenic potential in community and health care settings.

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